Functional characterization approach to identify novel antimicrobial resistance genes using cosmid libraries from *Pseudomonas* spp.

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Introduction

Antimicrobial resistance (AMR) is a major global threat, with impacts comparable to the COVID-19 pandemic[1,2]. Among critical priority pathogens, *Pseudomonas* species, especially carbapenem-resistance-strains are of particular concern due to their intrinsic resistance mechanisms and capacity to acquire novel resistance genes. Despite their clinical importance, relatively few studies have characterized antimicrobial resistance genes (ARGs) in environmental *Pseudomonas* strains[3].

In this study, 33 *Pseudomonas* isolates from municipal wastewater in Southern Ontario were analyzed under carbapenem selection. While most strains showed multiple resistance genes, 28 lacked known resistance genes to carbapenems including **β-lactamases and carbapenemases**.

This highlights wastewater as a rich, underexplored reservoir of AMR, with the potential for the discovery of novel resistance genes.

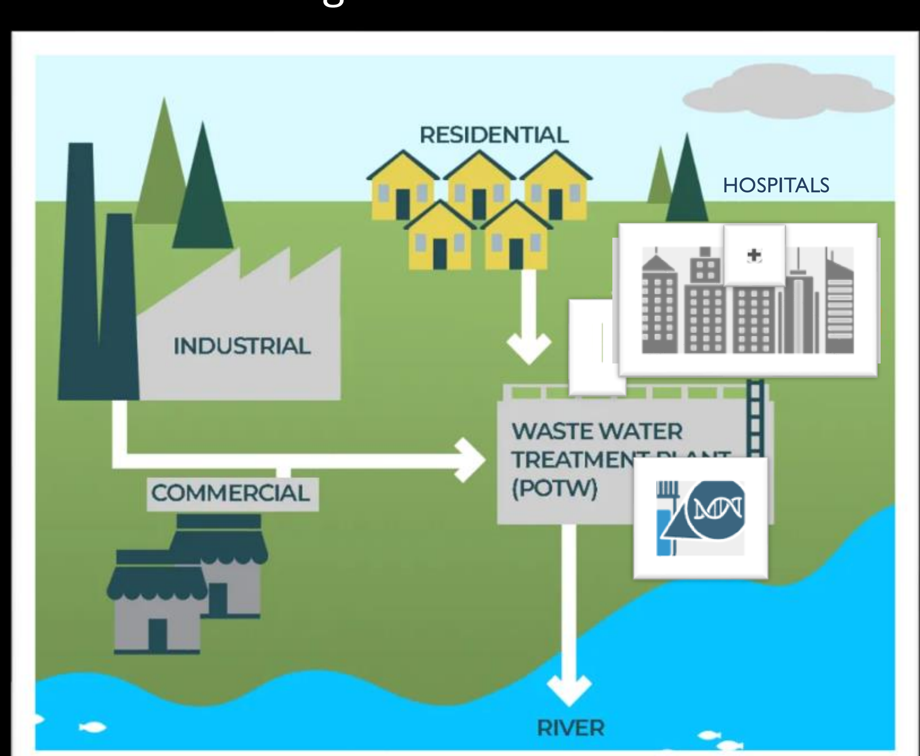

Figure 1. Wastewater as a reservoir of ARGs, and a potential source for the discovery of key resistance genes like carbapenemases.

Objectives

- Construct *Pseudomonas*-based genomic libraries to capture ARGs by isolating resistant clones.
- Generate transposon mutant cosmid clones to enable gene disruption and functionally screen to locate genes contributing to carbapenem resistance.
- Assess and characterize novel ARGs using sequencing and bioinformatic analysis and evaluate their potential environmental and clinical impact.

Conclusion

A TnPhoA insertion has been identified in a previously uncharacterized gene. This disruption was associated with a loss of meropenem resistance in the clone, suggesting that the interrupted gene plays a role in antimicrobial resistance. These findings suggest that this approach can be used to uncover novel, clinically relevant resistance genes.

Methods

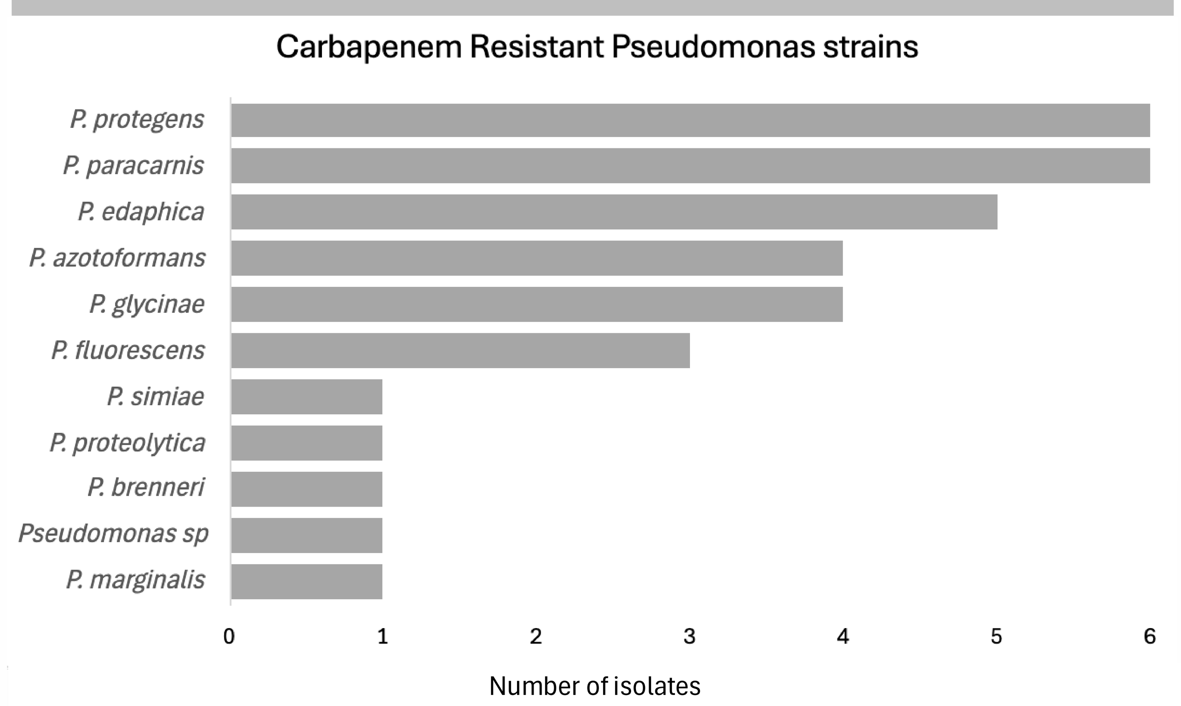

Figure 2. Abundance of *Pseudomonas* strains in Wastewater. Species assignation based on genomic Average Nucleotide Identity (ANI) ≥ 95.5% to the GTDB[4].

Genomic Library Construction

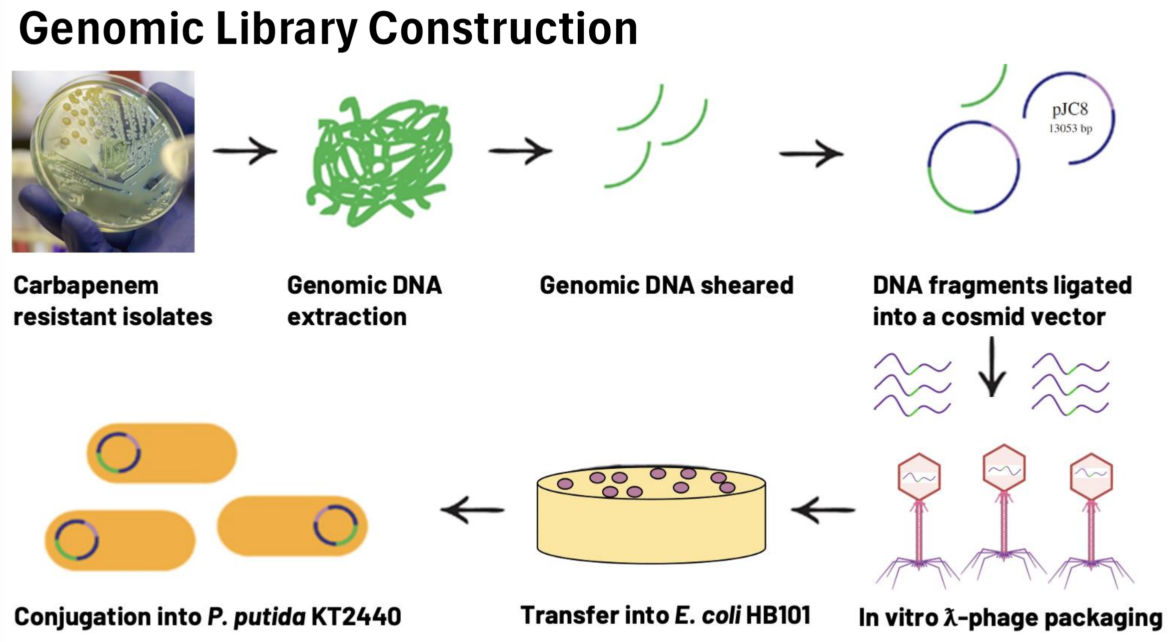

Figure 3. *Pseudomonas* genomic library construction using packaging extracts, and *P. putida* KT2440 as a recipient host.

Transposon Mutagenesis

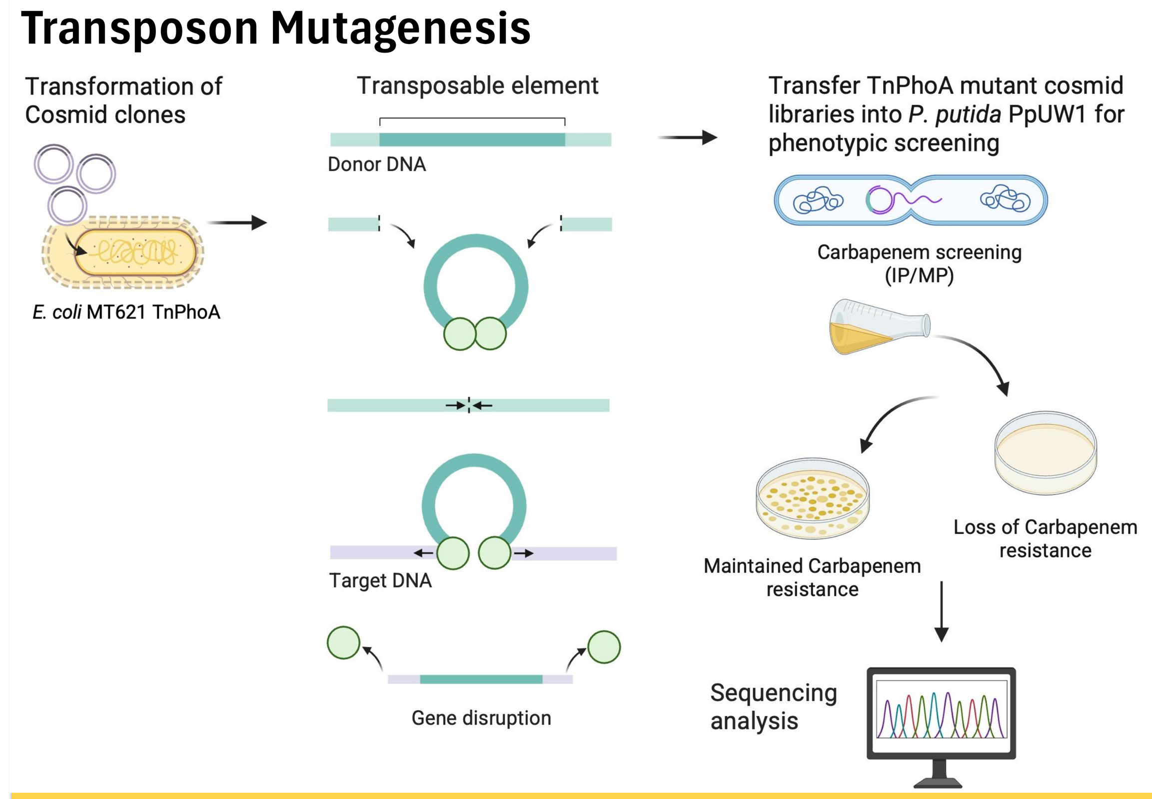

Figure 4. Transposon mutagenesis workflow to generate insertional disruptions in cosmid clones. Mutant libraries are transferred to *P. putida* to perform functional screening under meropenem selection to pinpoint genes contributing to carbapenem resistance.

Acknowledgements

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Results

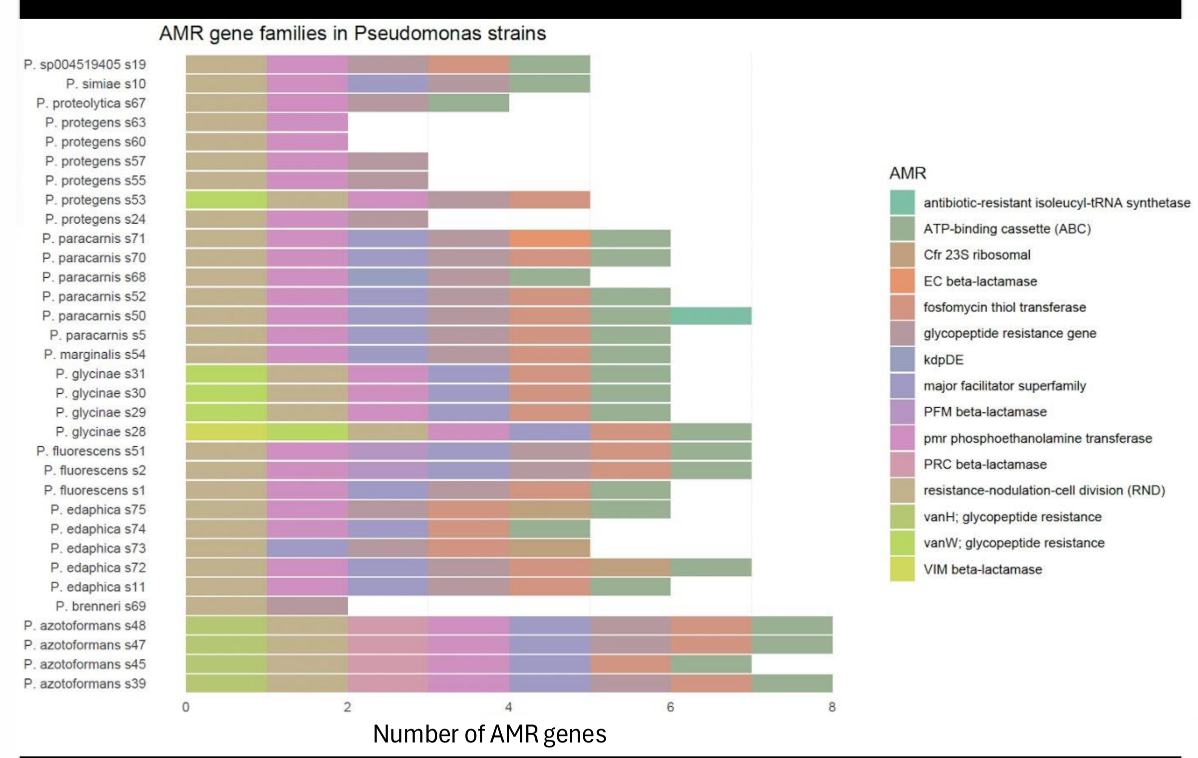

Figure 5. Identification of AMR gene families in *Pseudomonas* (CARD[5]). Among 33 strains, only *P. fluorescens* S2, *P. fluorescens* S51, and *P. glycinae* S28 carried carbapenem *beta*-lactamase genes (PFM, PRC and VIM).

TnPhoA Insertion Map

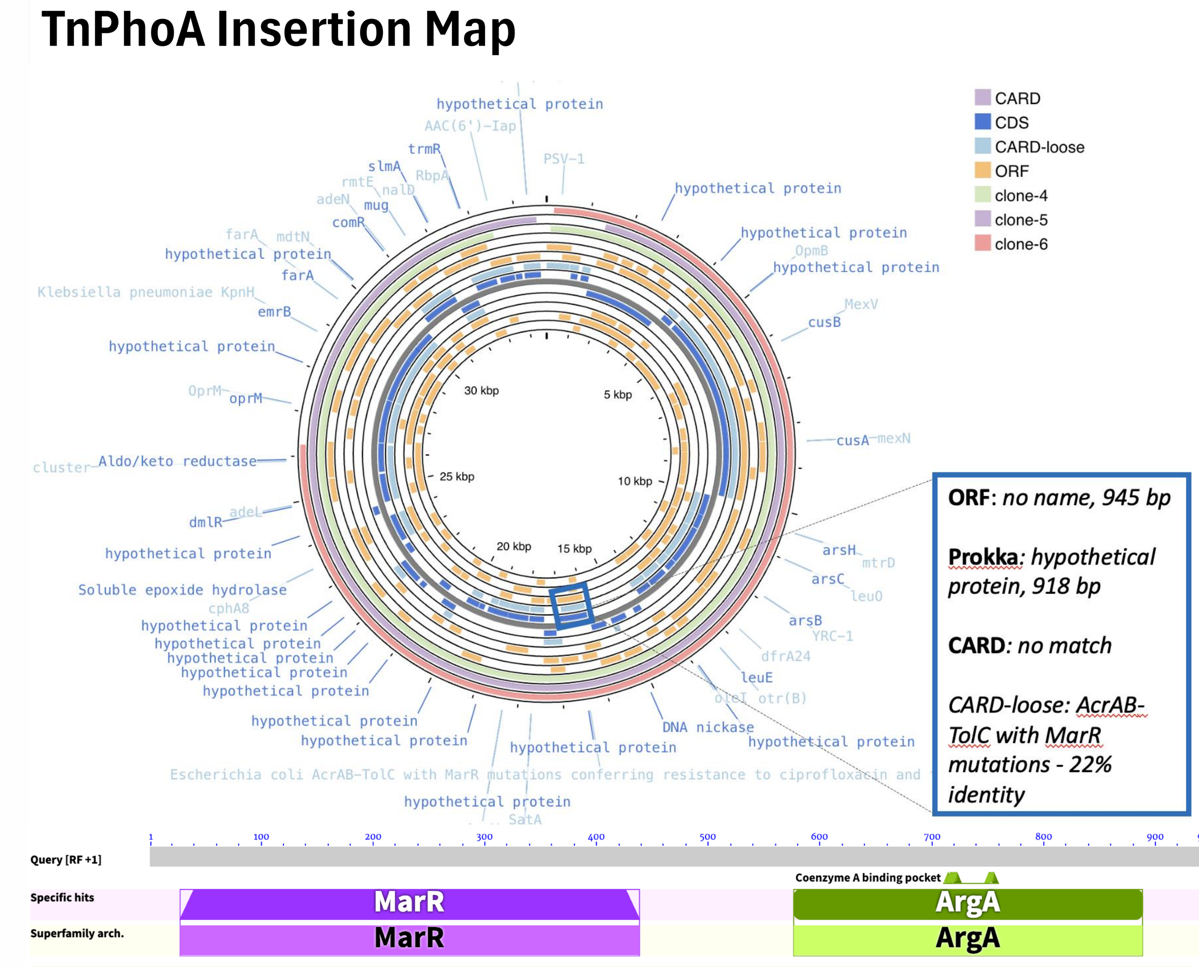

Figure 6. Mutant cosmid clone unable to grow on meropenem showed a TnPhoA insertion into an unidentified ORF. Non-strict mapping with CARD[5] and CD database[6] provided insights of possible domains related to transcriptional regulation (MarR). Sequencing performed with MinION ONT[7]. TnPhoA mapping was done using Minimap2[8]. Circularized for visualization.

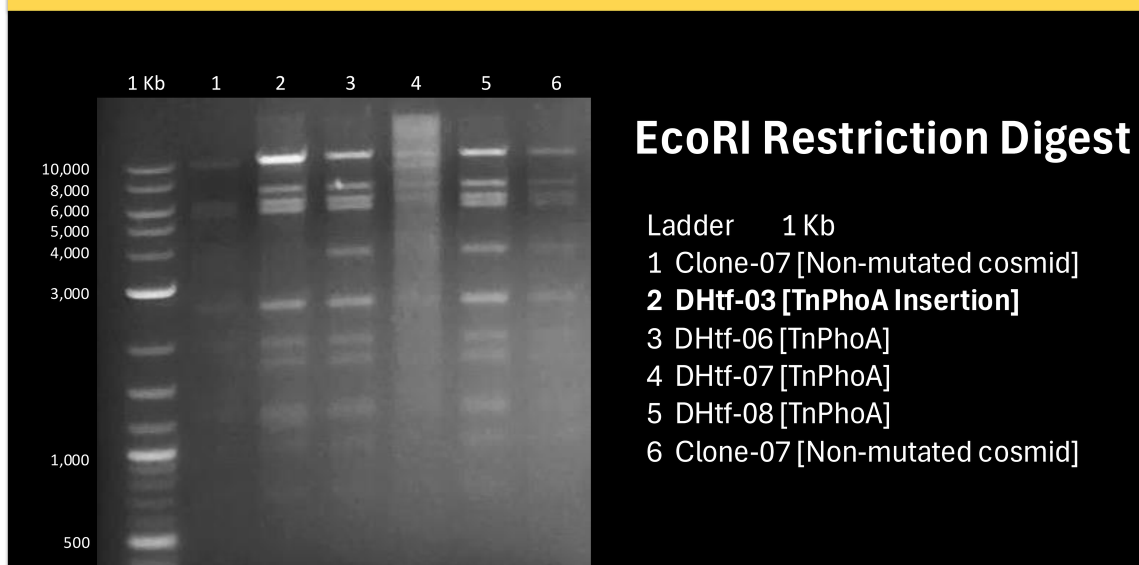

Figure 7. The TnPhoA insertion was detected by enzymatic restriction digest using EcoRI. A missing band of about 4 Kb in mutant DHtf-03 (lane 2), and an increased signal above the 10 Kb band suggests an inserted 7 Kb fragment at that position, which corresponds to the transposon length.

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References